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jc690 U.S. PTO

UTILITY PATENT APPLICATION TRANSMITTAL
 (only for continuation and divisional
 applications under 37 CFR 1.53(b))

Docket No.: P-IM 4082

Prior Application Info

Examiner: Y. Ryan

Group/Art Unit: 1641

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Steven Hsieh

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This is a request for filing a
 X continuation divisional

application under 37 CFR 1.53(b), of pending prior application
 serial no. 08/482,454, filed June 6, 1995, (list only immediate
 prior application).

Title: METHOD FOR INCREASING HDL CHOLESTEROL LEVEL
 Inventor(s) (full name of each inventor): Deborah Y. Kwoh,
 Steven W. Brostoff and Dennis J. Carlo

No abandonment of, or termination of proceedings, has occurred
 in the above-identified prior application.

1. X An application based on the prior application as
 filed and containing no new matter is enclosed,
 consisting of:
 1 page application cover sheet
 9 pages of specification (includes claims and
 abstract)
 0 sheets of drawing(s).
2. X 3 pages of a copy of the oath or declaration
 filed on October 9, 1995, from prior
 application (37 CFR 1.63(d)), U.S. serial no.
 08/482,454, filed June 6, 1995, is enclosed.

09523033 031000

Figure 1

_____ filed under Sec. 1.34(a)

Initial Information Data Sheet

Inventors: Kwoh et al.

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Correspondence Information

Name Line One :: CAMPBELL & FLORES LLP
Address Line One :: 4370 La Jolla Village Drive
Address Line Two :: Suite 700
City :: San Diego
State/Province :: California
Country :: USA
Postal or Zip Code :: 92122
Telephone :: (858) 535-9001
Fax :: (858) 535-8949
E-Mail :: CColacino@candf.com

Application Information

Title Line One :: METHOD FOR INCREASING HDL CHOLESTEROL
Title Line Two :: LEVEL
Total Drawing Sheets :: None
Application Type :: Utility
Docket Number :: P-IM 4082

Representative Information

Registration Number One :: 31,815
Registration Number Two :: 34,949
Registration Number Three :: 30,806
Registration Number Four :: 38,701
Registration Number Five :: 36,933
Registration Number Six :: 39,200
Registration Number Seven :: 38,444
Registration Number Eight :: 37,915
Registration Number Nine :: 41,029
Registration Number Ten :: 44,048
Registration Number Eleven :: 43,947
Registration Number Twelve :: 45,201

Continuity Information

This application is a :: Continuation, of
> Application One :: 08/482,454
Filing Date :: June 6, 1995

jc525 U.S. PRO
09/523033

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DATE OF DEPOSIT: **March 10, 2000**

Steven Hsieh
Printed Name of Person Mailing Paper or Fee

Steven T. Seal
Signature of Person Mailing Paper or Fee

METHOD FOR INCREASING HDL CHOLESTEROL LEVEL

This invention relates generally to the field of immunotherapy and, more specifically, to methods of stimulating an immune response to cholesteryl ester transfer protein (CETP).

BACKGROUND OF THE INVENTION

Blood cholesterol levels have long been thought to correlate directly with risk of atherosclerotic cardiac disease, the leading cause of heart attacks. More recently, it has been appreciated that blood cholesterol is actually composed of two primary forms: the high density lipoproteins (HDL) and low density lipoproteins (LDL). Rather than being associated with the disease risk, high HDL levels are apparently inversely predictive. In fact, studies have now indicated that HDL has a direct action in protecting against atherosclerosis and may even promote atherosclerosis plaque regression.

Numerous factors are involved in regulating the level of cholesterol in the body. Cholesteryl ester transfer protein (CETP) is an enzyme responsible for transporting cholesterol esters (CE) from HDL to very low density lipoproteins (VLDL) and LDL. VLDL's are eventually converted into LDL. CETP accelerates specifically the exchange of lipid components between pro- and anti-atherogenic lipo protein fractions. In particular, there is a strong inverse correlation between the levels of CETP in the plasma and the levels of HDL cholesterol. CETP activity levels are elevated in individuals suffering from dietary or genetic hypercholesterolemia. Increased levels of CETP activity result in lowered levels of HDL. In contrast, individuals with deficiencies in CETP activity due to mutations in the CETP gene have markedly elevated HDL levels.

The immune systems of higher organisms developed as a means for protecting the individual against invasion by deleterious foreign materials such as viruses, bacteria and parasites. Cells of the immune system are able to distinguish between materials from the individuals own body (termed "self" materials) and foreign material, or antigens. When foreign material enters the body, the immune system mounts a response. Antibodies that specifically recognize and bind to the foreign material are produced (the antibody or humoral response.) In addition, T cells are mobilized to repel the foreign substance (the T cell or cellular response.) Materials which are recognized as self do not normally stimulate such responses except in certain pathological conditions, primarily auto-immune disease. Even where the presence of an endogenous protein is itself deleterious, the immune system cannot serve as a regulator if the material is recognized as self.

Because of HDL'S potentially beneficial effect in preventing atherosclerosis, there exists a need for methods which can be used to increase its level in the serum. Such methods should ideally be specific and reliable and involve as little invasion of the body as possible. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method for increasing HDL cholesterol in a mammal by stimulating an immune response that inhibits the function of CETP. Such an immune response can be induced by immunizing with CETP or fragments of CETP (together termed "CETP Peptides") which contain an epitope capable of stimulating such a response. The peptides can be conjugated to a carrier, such as Keyhole Limpet Hemocyanin (KLH) or ovalbumin, in

order to increase immunogenicity. Adjuvants can also be administered.

In one embodiment, the fragments of CETP used to raise the antibody response are about ten to twenty amino acids in length and contain sequences homologous to the sequence in rabbit or human CETP.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a means to utilize the body's own immune system to lower CETP levels, thereby increasing the level of beneficial HDL cholesterol. The invention provides an effective method of raising HDL in the blood or more specifically, the serum. By utilizing the body's own immune system to increase HDL levels, the invention avoids the problems associated with the repeated administration of drugs, which have undesirable side effects.

According to the present invention, CETP peptide is administered to an appropriate individual in such a manner as to elicit an anti-CETP immune response. The CETP can be chosen to contain an epitope capable of stimulating an antibody or humoral response. Alternatively, the CETP can stimulate a cellular response, or other immune response. CETP peptides can be elected to contain B cell epitopes, sequences capable of stimulating the production of antibodies that specifically recognize and bind to the epitope. Alternatively, CETP peptides can be chosen which stimulate a T cell or more general immune response.

Individuals exhibiting, or at risk of exhibiting, low serum levels of HDL cholesterol are particularly appropriate for such treatment. Serum HDL levels can be determined using methods well-known in the

Serum HDL of less than about 30-35 mg/dl is considered low. Subjects exhibiting a serum HDL level below this

The protein or peptide to be administered can be all or part of the CETP protein, so long as the protein or peptide contains a B cell and/or T cell epitope. As used herein, "CETP peptide" is intended to include both the full length CETP amino acid sequence as well as fragments thereof. The peptides can have a sequence corresponding to or homologous to a mammalian CETP sequence. It will be appreciated that the peptide can differ from the native sequence to some extent so long as it is capable of inducing antibodies that inhibit the activity of CETP.

Because CETP and its peptide derivatives may be recognized as "self" antigens, carriers can be used to increase their immunogenicity. Such carriers are well known in the art and include, for example, such compounds as Keyhole Limpet Hemocyanin (KLH), ovalbumin and Diphtheria toxoid (Wako BioProducts). The CETP peptides can be conjugated to such carriers by methods well-known

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(SEQ ID No.: 1)

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(SEQ ID No.: 3)

The first peptide (SEQ ID 1) is taken from the Human CETP peptide sequence (residues 131-142 without signal peptide) from Smith and Barakat, Med. Sci. Res., 21:911-912 (1993), which is incorporated herein by reference. The second peptide (SEQ ID 2) is the corresponding rabbit sequence and differs by only 3 amino acids from the human.

The third peptide (SEQ ID 3) is common to both human and rabbit and is an epitope recognized by anti-CETP-monoclonal antibody which is neutralizing. Tall, A.R., J. Lipids Res., 34:1255-1257 (1993).

The peptides were conjugated to ovalbumin by the procedure of Current Protocols in Molecular Biology, supra. Of four New Zealand White rabbits, approximately four months of age, two were injected intramuscularly with 100 micrograms of the ovalbumin-conjugated human peptide (Seq. ID No.: 1) and CFA in PBS saline and two were injected with the equivalent human/rabbit peptide (Seq. ID No. 3). The animals were boosted twice at one month intervals with with the same peptides in IFA..

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

1. A method of stimulating an immune response to increase HDL cholesterol in a mammal exhibiting low levels of serum HDL comprising administering to said mammal a composition comprising an immunogenic epitope of CETP.

3. The method of claim 1, wherein said composition is a peptide.

5. The method of claim 3, wherein said peptide is:

6. The method of claim 1, wherein said composition comprises a carrier.

8. The method of claim 1, wherein said composition is administered with an adjuvant.

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The present invention provides a method for increasing HDL cholesterol in a mammal by stimulating an immune response that inhibits the function of CETP. Such an immune response can be induced by immunizing with CETP or fragments of CETP (together termed "CETP Peptides") which contain an epitope capable of stimulating such a response. The peptides can be conjugated to a carrier, such as KLH or ovalbumin, in order to increase immunogenicity. Adjuvants can also be administered.

COPY

[illegible]

I believe I am an original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD FOR INCREASING HDL CHOLESTROL LEVEL, the specification of which

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

Kwoh, et al.
Serial No.: 08/482,454
Filed: June 6, 1995
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willful false statements may jeopardize the validity of the application or any patent issued thereon.

We hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

CATHRYN CAMPBELL, Registration No. 31,815; CHRISTINE BELLAS, Registration No. 34,122; SUSAN M. PERKINS, Registration No. 36,405; RICHARD J. IMBRA, Registration No. 37,643; PAUL C. STEINHARDT, Registration No. 30,806; GREGORY R. HOOK, Registration No. 38,701; DAVID SPOLTER, Registration No. 36,933; DAVID A. GAY, Registration No. 39,200; BARRY S. WILSON, Registration No. 39,431; and GREGORY P. EINHORN, Registration No. 38,440. Direct all telephone calls to Cathryn Campbell at telephone no. (619) 535-9001.

Address all correspondence to:

CATHRYN CAMPBELL
Campbell and Flores
4370 La Jolla Village Drive
Suite 700
San Diego, California 92122

Full name of first inventor: DEBORAH Y. KWOH

Inventor's signature: Deborah Y. Kwoh

Date: 9/21/95

Residence: Carlsbad, California

Citizenship: United States

Post Office Address: 2404 Jacaranda Avenue
Carlsbad, CA 92009

Variable	Mean	SD	Min	Max
Age	31.2	4.5	25	45
Gender	0.5	0.5	0	1
Marital status	0.3	0.5	0	1
Education	12.5	1.5	10	15
Income	1500	500	1000	2500
Occupation	1.2	0.8	1	5
Health status	0.8	0.2	0.5	1
Stress level	3.5	1.5	1	5
Life satisfaction	4.2	1.2	3	5
Resilience	2.8	1.0	1	4
Optimism	3.8	1.0	2	5
Gratitude	4.0	1.0	3	5
Self-efficacy	3.5	1.0	2	5
Emotional stability	3.2	1.0	2	5
Empathy	3.8	1.0	2	5
Prosocial behavior	3.5	1.0	2	5
Altruism	3.2	1.0	2	5
Helping behavior	3.8	1.0	2	5
Volunteering	3.5	1.0	2	5
Community involvement	3.2	1.0	2	5
Civic participation	3.8	1.0	2	5
Political engagement	3.5	1.0	2	5
Social responsibility	3.2	1.0	2	5
Environmental awareness	3.8	1.0	2	5
Sustainability	3.5	1.0	2	5
Ethical behavior	3.2	1.0	2	5
Moral reasoning	3.8	1.0	2	5
Integrity	3.5	1.0	2	5
Honesty	3.2	1.0	2	5
Trustworthiness	3.8	1.0	2	5
Reliability	3.5	1.0	2	5
Accountability	3.2	1.0	2	5
Responsibility	3.8	1.0	2	5
Compassion	3.5	1.0	2	5
Kindness	3.2	1.0	2	5
Generosity	3.8	1.0	2	5
Openness	3.5	1.0	2	5
Conscientiousness	3.2	1.0	2	5
Neuroticism	3.8	1.0	2	5
Extraversion	3.5	1.0	2	5
Agreeableness	3.2	1.0	2	5
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Extraversion	3.2	1.0	2	5
Agreeableness	3.8	1.0	2	5
Conscientiousness	3.5	1.0	2	5
Neuroticism	3.2	1.0	2	5
Extraversion	3.8			

Inventor's signature: *[Signature]*

Carlsbad, CA 92009

Inventor's signature: Dennis Carter

Post Office Address: Rancho Santa Fe, CA 92067